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TRANSACTIONS

Biochemical Studies on "Miso", Fermented Soy-bean Paste.

Part II. Influence of Temperature upon the Ripening of "Miso".

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INTRODUCTION

It is well known that "koji" fungus and micro-organisms play the important rôle in the fermentation of "miso", and it is natural that the temperature during fermentation has also a great influence upon the ripening of it.

We know further that the optimum temperature[1][2][3] of the enzymes contained in "koji" fungus lies between 37° and 55°. T. Nishimura[4] described the general characteristics of yeast and bacteria concerning the ripening of "miso" and "tamarimiso". T. Akagi, I. Nakajima and K. Tsugane[5] studied the micro-organisms of "hatchomiso", and still later, Y. Ishimaru[6] reported on the behaviour of bacteria of "shoyu". From these observations the optimum temperature for the active propagation of yeast and bacteria was found to be within the range of 25~40°.

O. Kellner and his coworkers[7] studied the chemical change of "inakamiso" by analyzing the samples taken from the upper part of a large fermenting tank at four different periods during 150 days of fermentation.

Y. Sakurai[8] analysed "sendaimiso" in order to see the chemical changes of the chief components which are essential for nutrition, taking samples from different tanks, fermented at different periods.

The present investigation was undertaken to ascertain to what extent the ripening of "miso" is influenced by the temperature, taking the action of enzymes and micro-organisms into consideration.

For this purpose the raw materials of "miso" were kept for ripening at different temperatures and from time to time samples were taken for analysis

in order to determine the chemical changes of the chief components, such as proteins, carbohydrates, organic acids etc.....

The results are given in the following :

Experimental

The samples used for analysis were prepared in the following way :

(1) 3.0 kg. (4.1 L. by volume) of soy-beans were first soaked in water for five minutes, washed in a bamboo basket and again soaked in an equal volume of water (11～12°) for 15 hours, whereby the weight increased to 7.0 kg. and the volume to 9.2 L.. Then the same volume of water was added and boiled for 5.5 hours. This operation was followed by the steaming of the beans in a Koch steam sterilizer for about an hour and after putting out the fire, it was left standing for 12 hours, in consequence of which the weight increased to 8.36 kg. and the volume to 9.4 L.. Finally the beans were mashed and used as a material for preparing the experimental samples.

(2) Each 557 g. of boiled soy-beans (moisture 68.53%) prepared in the above stated manner, 100 g. of rice "koji" (moisture 24.45%) and 80 g. of salt were well mixed and fermented in a number of beakers, which were carefully covered with paraffin paper and pressed under glass lid.

The compositions of soy-beans, boiled soy-beans and rice "koji" were given in the following Tables (Table I and II).

Table I.—*Chemical Composition of the Raw Material* (%).

Composition	Water	Total-N	Protein-N	Amino-N	NH_3-N
Soy-beans	10.06	6.03	5.58	0.16	0.009
Boiled Soy-beans	68.53	2.25	2.00	0.13	0.018
Rice "koji"	24.45	0.71	0.61	0.19	0.012

Composition	Reducing sugar (as glucose)	Non-reducing carbohydrate (as starch)	Free acid (as lactic)
Soy-beans	0.68	18.71	0.02
Boiled Soy-beans	0.42	3.38	0.45
Rice "koji"	17.50	41.50	0.29

Table II.—*Chemical Changes* during the Steeping and Boiling of Soy-beans* (%).

	Water	Total-N	Protein-N	Amino-N
Soy-beans	10.06	6.03	5.58	0.16
Beans soaked for 15 hrs. (with ext.)	——	6.02	5.43	0.17
Beans boiled for 2.5 hrs.	——	5.75	5.12	0.10
Extract of the beans	——	0.25	0.10	0.09
Beans boiled for 5.5 hrs.	——	5.69	4.87	0.12
Extract of the beans	——	0.36	0.17	0.09

	Ammoniacal-N	Reducing sugar (as glucose	Non-reducing carbohydrate (as starch)	Free acid (as lactic)
Soy-beans	0.009	0.68	18.71	0.02
Beans soaked for 15 hrs. (with ext.)	0.016	1.15	17.18	1.24
Beans boiled for 2.5 hrs.	0.020	0.54	15.07	0.79
Extract of the beans	0.003	1.42	2.20	0.17
Beans boiled for 5.5 hrs.	0·022	0.47	11.57	0.46
Extract of the beans	0.004	1.59	3.83	0.17

* Calculated on the assumption that soy-beans contain 4.61% ash.

For analysis 30 g. of each sample was filled up to 250 cc. with water, and filtered after shaking for an hour at room temperature. The filtrate was used for analysis, applying Van Slyke's method for amino-nitrogen, Bertrand's method for reducing sugars and acidimetry for free acids using phenolphthalein as indicator.

From the above table it can be seen that during the steeping there was a tolerable increase in reducing sugar and free acids, a decrease in protein nitrogen and non-reducing carbohydrates and a slight increase in amino and ammoniacal nitrogen.

As to the chemical changes of boiled beans, it is noticeable that 3/4 of the reducing sugars were found in the extract after 2.5 hour's boiling, there was also marked decrease of free acids in the beans after 5.5 hour's boiling.

After mixing the raw materials as already described, the following samples for analysis were taken :

I. Samples fermented at 55°, taken at the 4, 8, 12 and 40th day respectively.
II. " " " 35°, " " " 10, 20, 30 " 60th " "
III. " " " 27～32°, " " 15, 30, 45 " 70th " "
IV. " " " 25°, " " " 15, 30, 45 " 70th " "
V. " " " 15～25°, " " 20, 40, 60 " 80th " "

The total nitrogen was determined by Kjeldahl's method, protein nitrogen by Stutzer's, amino-nitrogen by Van Slyke's, ammoniacal nitrogen by Wurster's, reducing sugar by Bertrand's and free acids by acidimetry, using phenolphthalein as indicator. The results are shown in the following table :

TABLE III.—*Chemical Changes of "miso" during Fermentation (%).*

Sample I fermented at 55°

Duration of fermentation (days)	Total-N=100				Reducing sugar* (as glucose)	Non-reducing carbohydrate* (as starch)	Free acid* (as lactic)
	Protein-N	Amino-N	NH_2-N	Rest-N			
0	86.8	12.7	1.2	(−0.7)	4.72	9.23	0.64
4	53.0	21.8	2.7	22.5	8.28	5.30	1.14
8	46.5	22.5	3.2	27.8	8.57	4.50	1.40
12	45.0	22.2	3.0	29.8	8.20	4.48	1.68
40	40.9	19.7	2.9	36.5	7.63	4.42	1.65

Sample II fermented at 35°							
0	86.8	12.7	1.2	(−0.7)	4.72	9.23	0.64
10	57.9	24.3	4.2	13.6	8.53	4.88	0.92
20	39.3	25.5	4.4	30.8	8.42	4 49	2.03
30	38.9	26.4	4.7	30.0	8.30	3.75	2.04
60	38.9	21.6	6.6	32.9	8.15	3.69	2.01
Sample III fermented at 27～32°							
0	89.3	10.7	0.8	(−0.8)	4.90	9.78	0.53
15	48.0	20.0	2.8	29.2	8.38	5.55	1.05
30	43.8	24.5	4.9	26.8	8.55	4.64	1.26
45	37.8	27.1	5.2	29.9	8.49	4.06	1.41
70	38.3	28.4	6.0	27.3	8.35	3.89	1.70
Sample IV fermented at 25°							
0	86.8	12.7	1.2	(−0.7)	4.72	9.23	0.64
15	56.4	19.6	2.8	21.2	8.80	4.81	1.23
30	48.0	21.7	3.1	27.2	8.85	4.45	1.35
45	42.3	24.7	3.8	29.2	8.32	4.27	1.45
70	41.1	25.8	3.9	29.2	8.20	3.50	1.59
Sample V fermented at 15～25°							
0	86.8	12.7	1.2	(−0.7)	4.72	9.23	0.64
20	50.2	15.8	2.3	31.7	8.84	4.91	1.30
40	48.3	19.9	2.5	29.3	8.89	3.88	1.26
60	44.7	24.4	3.7	27.2	8.68	3.41	1.28
80	43.1	24.1	3.9	28.9	8.66	3.33	1.36

* Calculated on the assumption that the content of NaCl is 11.15%.

The above table shows that the decomposition of proteins proceeds quicker at a high temperature. The amino-nitrogen in each series increases with the decomposition of the proteins, but it shows the tendency to decrease when the decomposition comes to a stillstand. The increase of ammoniacal nitrogen corresponds to the decrease of amino-nitrogen. It is the highest in the samples kept at 35°, decreasing in the order of 25°, 15～25° and 55° respectively. Generally the decomposition of proteins and amino-acids proceeds very rapidly. In the samples fermented at a higher temperature the reducing sugars increase rapidly in the earlier stages, but those kept at a lower temperature produce finally more reducing sugars. On the other hand, the non-reducing carbohydrates decrease according to the increase of reducing sugars. This is partly due to the formation of free acids. Thus we see that, the reducing sugars of "miso" kept at 35° is comparatively lower in spite of the considerable decrease in the non-reducing carbohydrates.

The amount of free acids is the highest in the samples kept at 35°, followed by the those kept at 55°, 25° and 15～25° respectively.

The author has als tried to determine the influence of temperature upon the colour of "miso". For this purpose, aqueous extracts of various "miso" samples were compared colorimetrically with the standard solution of iodine in potassium iodide. The latter was prepared by dissolving 1 g. iodine in 100 cc. of 5% potassium iodide. 100 cc. of it were equivalent to 0.986 g. iodide

by the titration of N/10 thiosulphate. 1 cc. of this solution was now diluted with water to 500 cc. and used for determination.

30 g. of each sample were finely ground in a morter, adding a little distilled water and filled up to 250 cc., filtered after shaking well for one hour, whereupon the filtrate was compared with standard iodin solution using Duboscq's colorimeter. If 25.0 mm. of the standard solution is equal to 4.0 mm. of the solution of "miso", the degree of coloration derived is 2.4 mm., which was obtained by multiplying a reciprocal of the reading by 100. Table IV shows the relation between the temperatur and the colour of "miso":

TABLE IV.

Material	Days of fermentation	Reading of colorimeter	Degree of coloration	Material	Days of fermentation	Reading of colorimeter	Degree of coloration
Standard solution	——	25.0	4.0	Standard solution	——	25.0	4.0
Sample I fermented at 55°	0	41.0	2.4	Sample IV fermented at 25°	0	41.0	2.4
	4	16.0	6.3		15	30.0	3.3
	8	5.8	17.2		30	24.5	4.1
	12	4.9	20.4		45	20.8	4.8
	40	1.0	100.0		70	14.0	7.1
Sample II fermented at 35°	0	41.0	2.4	Sample V fermented at 15~25°	0	41.0	2.4
	10	23.0	4.4		20	28.0	3.6
	20	22.8	4.4		40	23.0	4.4
	30	5.0	20.0		60	20.0	5.0
	60	3.5	28.6		80	18.0	5.3
Sample III fermented at 27~32°	0	43.5	2.3				
	15	32.5	3.1				
	30	26.0	3.8				
	45	24.0	4.2				
	70	19.5	5.1				

We see from the above results that the samples fermented at 55° are deepest in colour. Those kept at 35° become darker after 20 days while those kept for 70 days at 27~32°, 25° and 15~25° respectively are either equal to that kept at 55° for one week or to that kept at 35° for three weeks.

Further, in order to ascertain whether the enzyme action of "koji" has much influence upon the coloration of "miso", half of the samples were steamed for 5 hours to destroy the enzymes, then the samples were covered with toluol (1 cm. in thickness) to prevent the propagation of bacteria. The result is shown in Table V:

TABLE V.—*Relation between the Colour of "miso" and the Enzyme Action of "koji".*

Material	Reading of colorimeter (mm.)
Standard solution	25.0
Mixed sample	35.9
"Miso" steamed for, 2.5 hours	7.4
"Miso" steamed for, 5.0 hours	5.1

	Days of fermentation	"Miso" unsteamed	"Miso" steamed for 5.0 hours		Days of fermentation	"Miso" unsteamed	"Miso" steamed for 5.0 hours
Sample I′ fermented at 55°	0	35.9	5.1	Sample IV′ fermented at 25°.	0	35.9	5.1
	4	26.8	4.0		15	26.5	4.2
	8	22.5	3.2		45	24.1	3.4
	12	15.8	2.8		70	16.5	3.1
Sample II′ fermented at 35°	0	35.9	5.1	Sample V′ fermented at 15~32°	0	35.9	5.1
	10	28.2	4.2		20	27.8	4.4
	20	23.4	3.8		40	25.6	3.7
	30	18.5	3.2		80	17.8	3.2

As shown in the above table there is no remarkable change in the colour of samples steamed in the Koch sterilizer for 5 hours, while that of the samples without steaming changes considerably due to the enzyme action. This agrees well with the result indicated in Table IV. It indicates also that the coloration is much prevented by avoiding the free access of air.

In regard to the relation between the coloration and the change of nitrogenous compounds as shown in Table III, the residual nitrogen of the sample 55°, increases remarkably after the mixing of the raw materials, and after 20 days it becomes deeper in colour than any other series. The residual nitrogen of the sample kept at 35° for 20 days is somewhat larger than those kept at a lower temperature. In short, the grade of the colour seems to be correlated with any other nitrogenous compound than proteins, amino and ammonical nitrogen.

The decomposition of the proteins and non-reducing carbohydrates and the increase of amino-nitrogen and reducing sugars are the quickest at 55°, the optimum temperature for the enzyme action; the acid formation is somewhat higher at 35°, due to the favourable growth of bacteria at that temperature. These observations show that the fermentation of "miso" is much accelerated by the enzyme action of "koji".

Summary

In order to ascertain the influence of temperature upon the ripening of "miso" five series of samples were kept for fermentation at 55°, 35°, 27~32°, 25° and 15~25° respectively. The samples taken at different intervals were analysed and at the same time the chemical changes during the steeping and boiling of the soy-beans were determined.

It has been confirmed that the fermentation at 55° proceeds most rapidly attaining the maximum after 8~12 days, at a lower temperature the fermentation proceeds more slowly, thus wanting 15~25, 20~30 and 40~60 days or ripening at 35°, 25° and 15~25° respectively. The amount of ammoniacal nitrogen and free acid was comparatively large at 35°.

It has also been observed that the ripening of "miso" is much accelerated

by the enzyme action of "koji". By the steeping of the soy-beans the increase of reducing sugars and free acids is noticeable and by boiling them some proteins, reducing and non-reducing carbohydrates are dissolved in water.

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ABSTRACTS
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(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part IX).—Several Pigments of yellow, Green and White Cocoons, (pp. 1239～52, Vol. 10, Nos. 10～12): By Masami Oku. (From the Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd., Ayabe, Japan.)

I. Water soluble pigment of yellow cocoon and the pigments of pale green cocoon (bamboo-grass-like color) and white cocoon.

As the author had already studied, a great part of pigments of the yellow cocoons (Ascoli piceno and Chinese No. 7) consisted of xanthophylls and small quantity of carotin on the way of oxidation, yet they had moreover water soluble pigment which was studied by the author and reported in this paper.

The 80% alcoholic extract of the yellow cocoon was divided into ether soluble (carotinoids) and water soluble fractions. From the latter the water soluble pigment was precipitated as lead salt by neutral lead acetate and gained as crude powder through digestion by dil. HCl and through neutralization by NaOH. This crude brownish yellow pigment showed many analogous reactions of flavonol glucoside and had its two absorption maximums at the frequency of 2700 and 3500. This glucoside was hydrolysed by H_2SO_4 into its aglucone and sugar, which was identified as glucose by osazone formation but the aglucone could not be identified owing to the shortage of material.

The pigments of bamboo-grass-like cocoon (Japan proper) was studied similarly as already given before and obtained two pigment fractions, namely, ether soluble and water soluble. The former showed free flavonol-like reaction as bombycetin and the latter flavonol glucoside reaction as bombycin, both containing nitrogen as their component, but carotinoid could not be found in any fraction of the pigments.

As the 80% alcoholic extract under pressure of white cocoon (European No. 3) revealed pale greenish yellow color, it was, therefore, divided into ether soluble and water soluble fractions. Each fraction showed faintly flavonol reaction and the water soluble fraction was hydrolysed and the sugar component was identified as glucose by osazone formation.

II. Pale rouge pigment of Chinese rouge cocoons.

Chinese rouge cocoons, viz. Hankow, spherical yellow cocoon and three moulting variety contain rouge pigment in small quantity, which is tightly combined with silk fibre, leaving xanthophylls in extract, when the cocoon is digested with 80% alcohol and water under pressure. This rouge pigment was extracted ultimately from the fibre by alcohol and ether by preliminarily

treating the fibre (already treated with 80% alcohol) with hot 20% alcoholic potash (5% KOH). This pigment was shown to be strongly adsorbed by aluminium oxide and obtained in solution by chromatographic analysis and showed very analogous absorption spectrum with lutein.

[Appendix] *Pigments of wild cocoons of Theophila mandarina and Rhodinia fugax.*

By spectroscopic examination of the alcoholic extract of cocoon of Theophila mandarina, it showed neither the features of carotinoid nor flavonol derivatives, but characteristic to the cocoon, absorbing slightly the wave length shorter than 400 mμ.

The green pigment of cocoon of Rhodinia fugax (similar tinge of cocoon of Antheraea yamamai) was studied spectrometrically and determined to be identical with chlorophyll.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part X).—Relation between Fluorescence Colours of Cocoons and that of Natural Pigments of the Cocoons under Irradiation with filtered Ultra-violet Light, (pp. 1253~58, Vol. 10, Nos. 10~12): By Masami Oku. (From the Chemical Laboratory of Gunze Row Silk Mfg, Co. Ltd., Ayabe, Japan.)

Many authors had already studied the fluorescence colours of cocoons when irradiated with ultra-violet light from various stand points but no one has established a trustworthy criterion from the stand point of pigments of the cocoon. The author had already studied the natural pigments of the domestic cocoons and reported the results in former papers. This time the author detected same trustworthy relation between fluorescence colours of cocoon and that of natural pigments of the cocoon by some experiments and reported the results in this paper.

Experimental

Domestic cocoons irradiated with ultra-violet light showed following different fluorescence, viz.,

1. White cocoon (pure line): yellow, violet and intermediate.
2. Yellow cocoon (European, Chinese): bright yellow and dark yellow.
3. White cocoon of cross variety of green one and pure variety Seihaku (green) of Japanese proper: almost all of them showed bright yellow.

(1) Pigments of yellow cocoons are xanthophylls (lutein, violaxanthin), carotin (partially oxidized) and water soluble one. These xanthophylls and carotin irradiated with filtered ultra-violet light showed dark red~dark brownish red fluorescence when inspected as crystalline state, but they showed orange yellow~bright yellow fluorescence according to their concentration, when they were dyed on filter paper (this paper showed pale violet fluorescence) from their alcoholic solutions and inspected.

(2) Japanese proper var. Seihaku, (green cocoon) has two main pigments, viz. bombycin (glucosid) and bombycetin (aglucone of bombycin) as already studied by the author and reported them in my previous paper of part VII.

Bright yellow fluorescence colour of the green cocoon could not be removed when treated with conc. alcohol, but it could be removed when treated with hot 70～50% alcohol and thus treated cocoon showed contrarily pale violet fluorescence. Bombycin and bombycetin (both are brownish yellow by naked eyes) as well as isoquercitrin (isolated by the author from mulberry leaves and reported in part VIII) and quercetin showed deep red—deep reddish brown colour of fluorescence when inspected as crystalline state, but they showed bright yellow colour of fluorescence when they were dyed on the filter paper from their alcoholic solutions and inspected similarily.

(3) Cross variety of Japanese bamboo-grass-like colour coccon showed pale violet fluorescence when treated with hot 70～50% alcohol. Its pigments (flavonol- and flavonol glucoside-like substances) showed very similar colour of fluorescence as stated in the case of green cocoon var. Seihaku.

(5) All the pigments of domestic cocoons studied by the author showed the fluorescence colour bright yellow—orange yellow—brownish red—deep red—dark red, according to their concentration as stated above. As none of them showed violet colour of fluorescence, the origin of the yellow colour of fluorescence of the cocoons can be surely asked in their natural pigments but the origin of the violet colour of fluorescence of the cocoons must be asked in some other fractions than their natural pigments.

On the Natural Pigments of Raw Silk Fibre of the Domestic Cocoon (Part XI).—Summary from Part I to Part X and Discussion on Some Problems Concerning the Natural Pigments of Cocoons, (pp. 1258～62, Vol. 10, Nos. 10～12): By Masami Oku. (From the Chemical Laboratory of Gunze Raw Silk Mfg. Co. Ltd. Ayabe, Japan.)

Part I. The author isolated xanthophyll $C_{40}H_{56}O_2$ from yellow cocoon in crystalline state and found it to be physico-chemically combined with sericine.

Part II. Xanthophyll from mulberry leaves was isolated and found to be identical with that from yellow cocoon. Therefore, xanthophyll of yellow cocoon can be deemed as derived biologically from mulberry leaves, on which silk worms feed.

Part III. Xanthophyll both from yellow cocoon and mulberry leaves was purified according to R. Kuhn's method and obtained at last the purest substance which was identical with lutein (m. p. 193°) as given by R. Kuhn. Some notes on the isolation of xanthophyll were given there.

Part IV. Carotin was contained in yellow cocoons in minor quantity and found to be on the way of oxidation. Xanthophyll ester could not be found in them.

Part V. Violaxanthin $C_{40}H_{56}O_4$ was positively tested in the mother liquor of lutein.

Part VI. Fading of colour of yellow cocoon could be attributed to the

oxidation of its main colouring matter, xanthophyll. Yellow cocoon's odor too, was cleared up to arise from the oxidation product of xanthophyll.

Part VII. The pigments of Japanese green cocoon were studied and two new pigments, bombycin (glucoside) and bombycetin (aglucone of bombycin) was isolated, which was found to contain one atom of nitrogen and yet to show flavonol reaction. These two new pigments were presumed to have been synthesized biochemically in the body of silk worm from the components of mulberry leaves.

Part VIII. Isoquercitrin was isolated from mulberry leaves as their main flavonol glucosidal pigment but bombycin could not be found in any quantity.

Part IX. In water soluble fraction of alcoholic extract of yellow cocoon, flavonol glucosidal pigments were tested positively. From pure variety of white cocoon and crossed variety of bamboo-grass-like colour, too, flavonol-and flavonol glucoside-like pigments were tested positively. Rouge pigment of Chinese rouge cocoon was identified to belong to xantophylls. Pigments of Theophila mandarina was neither carotinoids nor flavonol derivatives but peculiar to it and that of Rhodinia fugax was identified to be chlorophyll.

Part X. All the natural pigment of domestic cocoons studied by the author showed the fluorescence colour yellow—orange—red, according to their concentration and were responsible for the yellow colour of fluorescence of the cocoon.

Discussion on some actual problems

(1) Unevenness of color of raw silk.

Pigments of yellow cocoon distribute unevenly in the cocoon layer and cause, therefore, the unevenness of color of raw silk. Generally speaking, carotinoid content of yellow cocoon is meager in the outer and inner layer and rich in the middle layer. But by silk reeling in filature, several cocoons different in position of the cocoon layer are reeled to one thread, so the different distribution of the pigment of cocoons cancels each other and appears to the naked eyes relatively evenly. But in practice we must take care to prevent the case in which we meet with the unevenness of color of raw silk, keeping in mind the uneven distribution of carotinoids in the yellow cocoon.

Not only in the case of yellow raw silk, but also in the case of white raw silk, we hear sometimes complaint of colour differences by silk conditioning. In this case the flavonol- and flavonolglucoside-like pigments as well as carotinoids, which is revealed by cross-breed, are responsible for it.

(2) Heredity of colour of cocoons.

Heredity of colour of coccoons follows generally the law of Mendelism but there are some exceptions. At present crossing are carried out regarding the colour of cocoons only to the naked eyes, so there occurs the case in which some unaccountable colour appears. The author wishes that my studies regarding the natural pigments of cocoon shall help in future the improvement

of cross-variety of silk worms.

(3) Colours of fluorescence of cocoons.

By means of improved heredity and acknowledgment of the importance of natural pigments of cocoon, the uniformity of fluorescence colour of the cross-breed must be succeeded and then we shall elevate the value of raw silk as an international merchandise still more.

(4) Growth of microorganisms on cocoons.

White cocoon layers as well as yellow cocoon have the sugar component as glucosidal pigments which serves as a source of carbohydrate to micro-organisms. But in practice they grow on the white cocoon easier than on the yellow cocoon, which fact can be explained to be due to the fact that the latter contains xanthophylls (a part of them is on the way of oxidation) which effect unfavourable conditions upon their growth.

(5) Bleaching of the yellow raw silk.

Yellow raw silk must be bleached before it is dyed and weaved. Several methods are employed for that purpose, but that of sodium peroxide (oxidation method) has been most popularily employed and recommended. Even in that case, a last trace of yellow tinge is difficult to decolourize and this is attributed to the existence of xanthophyll which is tightly combined with silk fibroin.

Darstellung des Harnstoffes aus Kohlensäure und Ammoniak (I. Mitteillung). (pp. 1333～44, Vol. 10, Nos. 10～12): von Matsuo TOKUOKA. (Taihoku Universität, Taiwan, Japan.)

Der Hauptzweck vorliegender Arbeit liegt darin, die physico-chemiche Bedingungen zur Herstellung des Harnstoffes aus Kohlensäure und Ammoniak systematisch aufzuklären.

In dieser Mitteilung wurde hauptsächlich die Herstellung von Ammoniumcarbamat aus Kohlensäure und Ammoniak untersucht. Dabei wurde auch der Dissoziationsdruck von Ammoniumcarbamat bei gewöhnlichen bzw. höheren Temperaturen ermittelt. Durch die Anwendung von Reaktionsisochore wurde die Reaktionswärme von Ammoniumcarbamat berechnet.

1. Die Kondensation des Gemisches von Kohlensäure und Ammoniak.

Diese Versuche wurde durch die dynamische Methode ausgeführt. Dabei hat die Beimischung des Wasserdampfes bis 1.5% immer die Kondensation des Gemisches beschleunigt. Die Vermehrung der festen Oberflächen im Reaktionsraum hat auch die Kondensation erleichtert.

2. (a) Der Dissoziationsdruck von Ammoniumcarbamat bei niedrigeren Temperaturen.

Temp. °C	Dis.-Druck (mm/Hg).	Temp. °C	Dis.-Druck (mm/Hg).
5,0	20,31	36,0	194,69
11,0	32,22	44,0	329,09
18,0	55,58	51,0	492,12
27,0	102,84		

Aus diesen Resultaten hat man die folgende Formel erhalten.

Aus den Zahlen, und zwar von 18, 36, 51°C, hat man folgende Formel erhalten, d.h.

$$\log P = \frac{-3287{,}609}{T} - 4{,}3651 \log T + 20{,}91844$$

2. (b) Der Dissoziationsdruck von Ammoniumcarbamat bei hoheren Temperaturen.

Es wurde mit dem speciell von dem Verfasser konstruierten Apparaten der Dissoziationsdruck ermittelt. Die ermittelte und die durch die oben angegebene Formel berechnete Werte sind folgende. Die Beide haben eine sehr gute Übereinstimmung gezeigt.

Temp. (°C)	Dissoziationsdruck von Ammoniumcarbamat (Atm).	
	Gefundene	Berechnete
72	1,85	1,909
88	4,25	4,428
100	7,50	7,537
110	11,40	11,405
120	16,25	16,855
130	——	22,729
140	——	34,493
150	——	47,924
160	——	65,427

3. Durch die Anwendung von Reaktionsisochore wurde die Reaktionswärme von Ammoniumcarbamat bei 5.0～51°C als +37, 223 kal. berechnet.

Studies on the Digestion of Food V.—On the Digestion of Synthetic Fats (3), (pp. 1～10): Sigeo SUZUKI. (Agr. College of Kagoshima, Japan.)

Nutritive Value of Canavanin (a new amino acid). (pp. 11～15): By M. OGAWA (Dept. of Nutrition, Tokyo Municipal, Hyg. Lab., Tokyo)

In the previous communication the author reported a nutritive value of a new amino acid called Canavanin. Although, the author reported the results of the first 40 days of the experiment. But, this present report is consisted of the results of the previous experiment which continued for 83 days, employing the same animals. From this experiment, the author obtained the following results :

(1) Canavanin was not poisonous or harmful substance.

(2) The animals which administered Canavanin had a flow of spirits, while the animals did not administered Canavanin were rather depression of

spirits, and this fact was conspicuous especially when the animals were young.

(3) The animals administered Canavanin were grew better and increased the body weight more rapidly than the animals which did not consume Canavanin. The amount of the consumption of the diet of the former was lesser than that of the consumption of the latter. This fact was conspicuously recognized up to a certain period, such as the body weight of the animals reached to about 100 grams.

From the above results, the author concluded that Canavanin was one of an important amino acid and it was an essensial for the growth, especially in the early life of the animals.

Untersuchugen über die Enzyme von Bombyx mori L. X. Mitteilung.—Über die Magenlipase. (1), (pp. 16～21): Von K. YAMAFUJI und Y. YONEZAWA. (Biochem. Institut der Landw. Abteilung der Kaiserl. Kyushu-Univ. zu Fukuoka, Japan.)

Als wichtige Verdauungsenzyme der Seidenraupe können wir vier Fermente aufzählen: Lipase, Amylase, Saccharase und Protease. In bezug auf das fettspaltende Enzym des Magensaftes hat im Gegensatz zu den drei letztgenannten noch niemand eingehende Untersuchungen angestellt, obwohl es von grosser Wichtigkeit ist, durch genaue Kenntnis der Eigenschaften der Magenlipase die Züchtung dieses Insekts zu verbessern.

Beim einfachen qualitativen Versuch der verschiedenen Enzyme von Bombyx mori durch Kawase[1] zeigt es sich, dass der Digestionssaft sowohl Olivenöl als auch Aethylacetat wenig spaltet, und ferner, dass das Blut keine Lipase enthält. Aber wie der eine[4] von uns kürzlich berichtet hat, lässt sich die lipatische Wirkung des Blutes mit der stalagmometrischen Methode quantitativ bestimmen. Auf Grund dieser Erfahrung haben wir uns nun mit einer weiteren Untersuchung beschäftigt, um durch Anwendung dieser Methode auf die Magenlipase die Verdauungsphysiologie der Seidenraupe weiter aufzuklären.

1) *Bestimmungsmethode.* Die Lipasewirkung wurde wegen der äusserst geringen Enzymmenge im Magensafte ausschliesslich durch die stalagmometrische Messung der Tributyrinhydrolyse beobachtet. Die Reinigung von Tributyrin und die Herstellung der gesättigten Tributyrinlösung wurden in der üblichen Weise[2] ausgeführt. Der Bestimmungsansatz bestand aus 50 ccm substratlösung, 2 ccm 2,5 n-Ammoniumchlorid-Ammoniak-Gemisch von pH= 8,6 und 1 ccm verdünntem Magensaft. Die Versuchstemperatur war 20° und die Reaktionszeit 10 Minuten. Wir benutzten stets den frisch gewonnenen Magensaft.[3] Als relatives Mass für die Lipasemenge wählten wir die Konstante des monomolekularen Vorganges, da die Reaktionsgeschwindigkeit, wie wir in der nachfolgenden Abhandlung[5] zeigen, der Konzentration des Enzyms direkt proportional ist.

2) *Einfluss der Wasserstoffionenkonzentration.*

Puffer: Ammonchlorid-Ammoniakgemisch.

pH	8,0	8,6	9,2	9,8	10,4	11,0
k.10^3	16,7	25,3	32,6	46,6	30,1	24,9

3) *Einfluss der Temperatur.*

Nach 10 Minuten Erwärmung der Reaktionsmischung auf verschiedene Temperaturen wurden 0,05 ccm konz. HCl zugesetzt und dann die Tropfenzahl bei 20° ermittelt. Die Anfangstropfenzahl wurde gesondert bestimmt.

Temp.	10°	20°	30°	40°	50°	60°
k.10^3	3,3	21,3	33,1	44,4	39,9	25,9

4) *Thermostabilität des Enzyms.*

Der Magensaft wurde vorher 60 Minuten lang auf die erforderliche Temperatur erhitzt und darauf der Spaltungsversuch nach dem üblichen Ansatz durchgeführt.

Erhitzungstemp.	Nicht erhitzt	40°	45°	50°	60°	70°
k.10^3	23,6	16,9	11,8	7,2	4,8	1,4
Rel. k	100	72	50	31	20	6

5) *Einfluss von Chinin und Atoxyl.*

Spaltungsansatz: 50 ccm Tributyrinlösung + 2 ccm Puffer + 1 ccm Giftlösung + 1 ccm 10fach verdünnter Magensaft.

Chinin-HCl (mg)	k.10^3	Rel.k	Atoxyl (mg)	k.10^3	Rel.k
0	21,0	100	0	11,8	100
2,5	19,9	95	2,5	10,8	92
5,0	18,7	89	5,0	9,6	81

6) *Aenderungen duch Hunger bei Larve.*

Hungerstunden	3	26	50	72
k.10^3	13,0	27,2	11,8	9,7

7) *Veränderungen mit dem Wachstum der Larve.*

Tage	IV. Lebensalter		V. Lebensalter			
	3	5	2	3	5	7
♀, k.10^3	—	—	21,3	31,6	53,2	42,1
♂, k.10^3	—	—	24,4	48,4	55,3	47,7
♀ ♂, Mittel	7,2	15,6	22,9	40,0	54,3	44,9

8) Unterschied zwischen den verschiedenen Rassen.

Um den Vergleich genau anzustellen, wurden alle Versuchstiere unter denselben Umständen gezüchtet.

V. Lebensalter Tage / Rasse	3		5		7	
	♀, k.10^3	♂, k.10^3	♀, k.10^3	♂, k.10^3	♀, k.10^3	♂, k.10^3
Japan-110-E	32,7	36,8	49,3	53,2	12,9	16,9
Japan-110-G	15,7	21,7	42,3	39,9	28,9	42,0
China-7-B	31,6	48,4	53,2	55,3	42,1	47,7
China-7-D	30,4	41,2	42,0	50,9	38,0	44,0
Europa-7-A	15,6	18,4	58,7	67,7	29,5	44,5
Europa-7-C	23,6	25,6	62,9	69,9	28,4	35,3

Zusammenfassung

1. Die Magenlipase der Seidenraupe reagiert im stark alkalischen Gebiet; sie hat ihr Wirkungsoptimum bei pH=9,8. Dieser Wert stimmt mit dem pH des Verdauungssafter überein.

2. Das Temperaturoptimum der lipatischen Tributyrinspaltung liegt bei 40°. Im natürlichen Milieu ist die Tötungstemperatur für die Lipase 45°, bei 70° wird das Enzym in 60 Minuten fast völlig verstört.

3. Chinin und Atoxyl vergiften die Lipase nur wenig. Nach eintägigem Hunger bei Larven wird die fettspaltende Wirkung recht viel stärker, danach aber allmählich schwächer.

4. Der Lipasegehalt des Magensaftes des Männchens ist immer höher als der des Weibchens. Mit dem Wachstum der Larve steigert er sich sehr bedeutend, nimmt aber gegen Ende des fünften Lebensalters ab. Diese Erscheinung scheint mit der Aufhäufung des Körperfettes zu tun zu haben. Der Unterschied zwischen der Lipasewirksamkeit der verschiedenen Rassen ist nicht deutlich.

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Chemical Studies of Japanese Coccidae. VIII.—On the Nitrogenous and organic Substance of Iceria purchasi Mask, (pp. 21～28): Michio Kawano and Ryunosuke Maruyama.

Carbohydrats of the Bulbs of Allium. IV.—Hydrolysis of Scorodose by the Enzymes, (pp. 29～33): Yoshijiro Kihara.

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A Systematic Study of Alcohol and Carbohydrate Oxidizing Bacteria isolated from Fruits, and a New Classification of the Oxidizing Bacteria. (pp. 50~60): Toshinobu Asai. (Agr. Chem. Laboratory, Imp. University of Tokyo)

Studies on the Amylase of Yeast. I.—Preparation of Amylase Solution from Pressed Yeast (1), (pp. 60~67): Kazuki Ono.

Studies on the Castor-bean Lipase. VI.—On a new preparation of highly active ricinus-lipase and its properties, (pp. 68~76): By Etsuo Takamiya. (Bioch. Laboratory, Depart. of Agr., Kyushu Imp. University, Fukuoka, Japan)

Willstätter says that in sleeping castor-seeds exists a spermatolipase which opt. pH is at 5.0, and on germination, the spermatolipase changes to a blastolipase possessing its opt. pH at 6.8, and that there exists no proenzyme (zymogen) on the ricinus-lipase. By a special treatment of sleeping castor-seeds with conc. (normal) inorganic or organic acids according to the author's method, the spermatolipase enzyme-unit (Ph L. E.) in seeds increases up to about its three times, and during that acid-treatment, the seed-protein is not at all hydrolysed and changes apparently to an acid-protein. From these experimental results, author suggested that most part of the ricinus-lipase in sleeping seeds exists in form of proenzyme combining with a seed-protein, and by the acid-treatment, the lipase (spermatolipase) is freed from the protein combined.

Starting with such an acid-treated seed, author obtained and purified a new preparation of the highest active ricinus-lipase with a 35% yield. Its enzyme-value (Ph L. W.) is 240 times to the original seed which enzyme-value (0.08 Ph L. W.) is almost nearer to that (0.075 Ph L. W.) used by Willstätter who obtained a 100 times active preparation to the original seed. Such a highest active preparation obtained by author is negative in Biuret–, Molish–, sterol-reaction and positive in Millon-reaction. Its opt. pH is 4.5~5.0 in acetate buffer solution and 5.6 in phosphate buffer solution which both opt. pH respectively is similar to that of spermatolipase in seeds. Its nitrogen and phosphor content respectively is 7.5% and 1.48%. And its specific characters newly discovered are that such an active preparation is easily inactivated by oxidising or reducing agents, and that its olive oil solution has a specific colour reaction with the $SnCl_3$-chloroform reagent used in the colour reaction of Vitamin A. The colour produced is indigo-blue changing slowly at standing to pink-violet, and after standing for a long time to dark redish brown colour.

Sample (0.1 c.c.)	chloroform	$SnCl_3$-chloroform reagent	Colour Produced
(1) highest active preparation in olive oil.	2 c.c.	2 c.c.	indigo-blue
(2) highest active preparation inactivated somewhat by aeration in olive oil.	〃	〃	indigo-blue with somewhat red
(3) highest active preparation inactivated at all by drying in air in olive oil.	〃	〃	dark redish-brown
(4) cod-liver oil.	〃	〃	brilliant blue
(5) olive oil.	〃	〃	dark redish-brown

The author's method to obtain the highest active preparation is as follows. Castor seeds (130 grs) are crushed and after addition of *N*-sulphuric acid solution (18 c.c.), kneaded for two hours at 25°C and extracted with ether (Merck) (600 c.c.) for one hour in a glass-stoppered flask filled with CO_2 gas which has been purified from reducing substances in it by being passed through water, conc. pyrogallol solution, pumice wetted with $CuSO_4$ solution and conc. H_2SO_4. Any purifing operations with a use of purified CO_2 gas must be carefully carried out by protection from oxidation of air. To the somewhat turbid ether extract obtained by centrifuging is added a same volume of petroleum ether (Merck), and this ether-petroleum ether solution is placed in a bottle sealed with a stopper at least over-night in an ice-box. After an upper clear solution separated has been sucked out, ether (800 c.c.) moreover is added to this residual solution containing precipitate, and shaked it for several times and left it over-night at room temperature, and finally an upper clear solution separated is sucked out. This operations is repeated once more. And the precipitate obtained by centrifuging the residual solution is washed with petroleum-ether by means of centrifugal separator and olive oil (100 c.c.) is added to it and stirred and centrifuged. The somewhat turbid olive oil fraction obtained is washed with ether and petroleum ether by means of centrifugal separator and 187.5 mg (Ph L. E. 900) active lipase is obtained. Since this lipase preparation is very unstable and easily inactivated by air, enzymic activity must be estimated after adding some olive oil for protecting from inactivation of air.